Protocol for SDS Page

1. Samples preparation

Concentrate the supernatant:

Mix samples with 5X loading buffer

Incubate 5 minutes at 95℃

Centrifuge samples at 5000rpm for 75s

2. Acrylamide gels preparation and running

Separating gel solution (10%):

Distilled water 1.08mL

Tris Hcl 1.5M pH 8.8 1.67 mL

50% glycerol 1.0mL

Acrylamide 1.25 mL

Ammonium persulfate 30 µL

TEMED 5 µL

Stacking gel solution :
Distilled water 2.0 mL

Tris Hcl 1.5M pH 8.8 0.5 mL

Acrylamide 0.5 mL

Ammonium persulfate 15 µL

TEMED 5 µL

Pour the separating gel solution. Leave enough space (~1.5 cm) on the top for stacking gel.

Overlay the separating gel with distilled water. Let the gel polymerize (~ 30 minutes).

Discard the water, and pour the stacking gel solution.

Carefully insert the comb, and avoid bubbles. Let the gel polymerize (~ 15 minutes).

Remove the tape and the comb, and then assemble on the running apparatus. Fill it with cathode buffer.

Rinse the wells with distilled water.

Load denatured samples. Put the running apparatus into the tank, and fill it with anode running buffer.

Run at 80V for 20min through stacking gel and run at 120V until the migration blue front has reached the end.

3. Gel staining – Coomassie blue

Submerge the gel in prestaining solution (acetic acid 10% + ethanol 50%). Shake until the migration blue front are clear.

Submerge the gel in the Coomassie blue stain solution and shake 30 minutes.

Submerge the gel in dH2O and shake until the protein bands are clear. Change the dH2O solution several times.